

Supporting Information

Bubier et al. 10.1073/pnas.0807309106

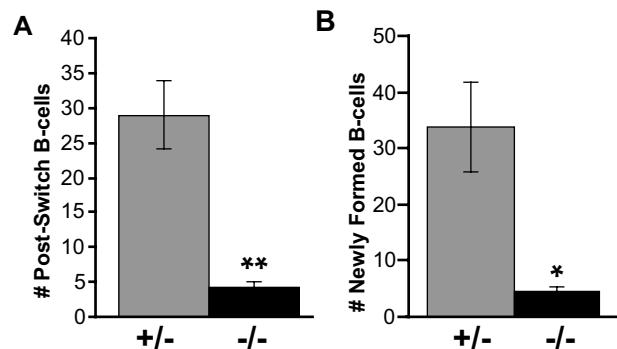


Fig. S1. IL-21R deficiency reduces the frequencies of newly formed and postswitch splenic B cells. Flow cytometric analysis of spleen cells shows that the abnormally abundant populations of postswitch (A) and newly formed (B) splenic B cells observed in *Yaa/IL21r^{+/−}* mice are significantly reduced in 16-week-old *Yaa/IL21^{−/−}* *Yaa* mice. Postswitch B cells were assayed by gating on viable AA4.1^{lo} B220⁺ IgM[−] IgD[−] viable cells. Newly formed B cells were identified as CD19⁺ CD21^{lo} CD23^{lo}.

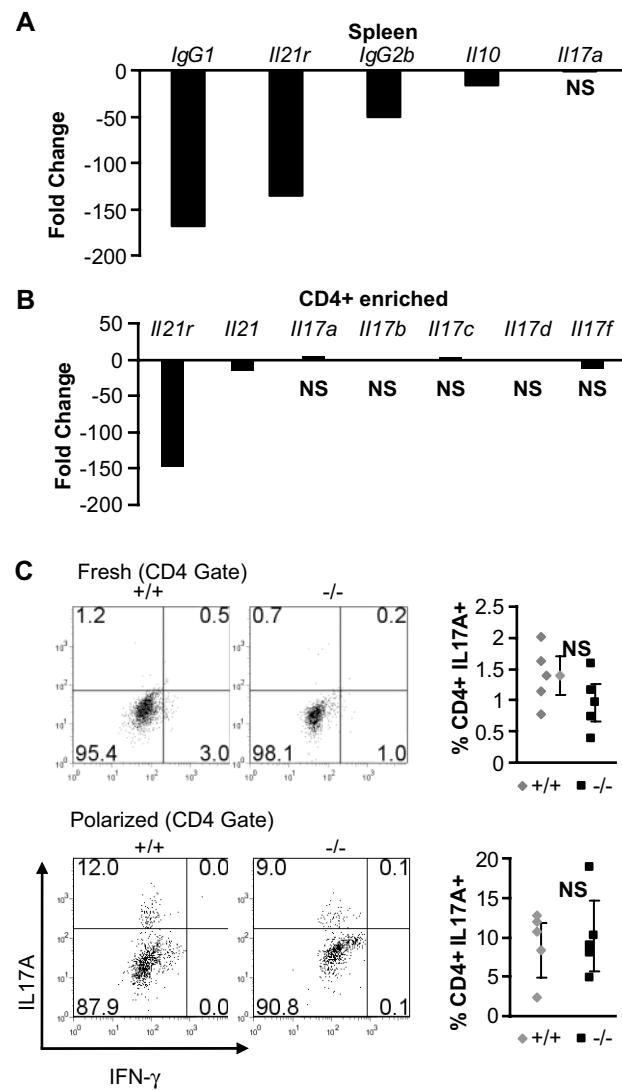


Fig. S2. Abrogation of autoimmune disease in IL-21R-deficient BXSB-Yaa mice does not correlate with the Th₁₇ cell population. (A) qPCR of splenic cDNAs from 16-week-old Yaa/*Il21r*^{+/−} and Yaa/*Il21*^{−/−} mice. (B) qPCR of cDNAs from CD4-enriched T cells from similarly aged groups. (C) Intracellular expression of IL-17 in splenic CD4⁺ T cells before and after Th₁₇ polarization. All values are significant at ≤ 0.05 unless indicated with NS; there were 3–5 mice per group. cDNAs for qPCR analysis were prepared from whole spleen or from CD4 T cells that were enriched by negative selection techniques using biotinylated antibodies against CD8, CD19, and CD11b followed by the magnetic separation of antibody-conjugated streptavidin microbeads (80% enrichment). Th₁₇ cell polarization was carried out as described [Hsu HC, et al. (2008) Interleukin 17-producing T helper cells and interleukin 17 orchestrate autoreactive germinal center development in autoimmune BXD2 mice. *Nat Immunol* 9:166–175]. Intracellular staining was performed after a 5-h stimulation with 50 ng/ml phorbol 12-myristate 13-acetate (PMA) and 750 ng/ml ionomycin as described by Hsu et al. by using the Intracellular Cytokine Staining kit (BD PharMingen) and antibodies to IL-17A (eBioscience).

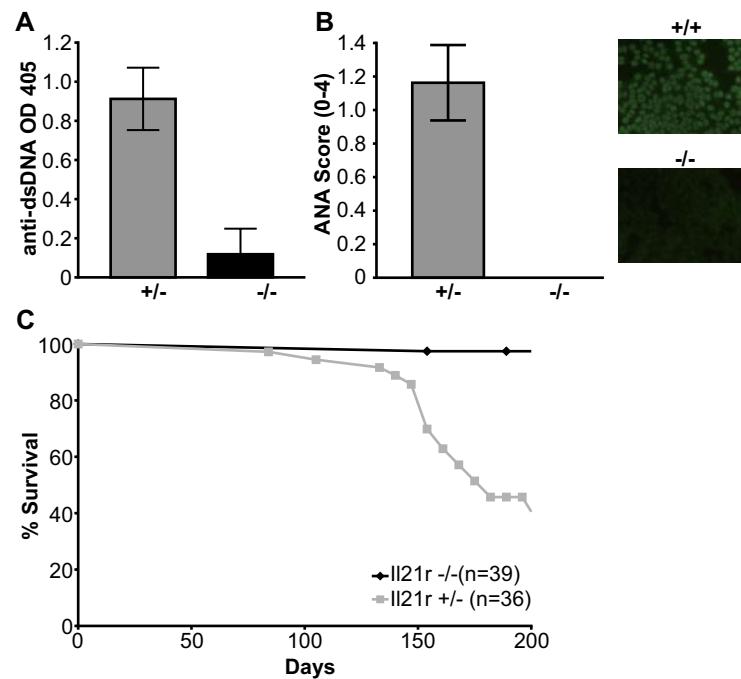


Fig. S3. Analysis of effect of IL-21-deficiency on BXSB-Yaa disease after 11 generations of backcross. Analysis of anti-double-stranded DNA (A) and ANA (B) of 7 *Il21r*^{+/−} and *Il21r*^{−/−} mice after 11 generations of backcross of the *Il21r*-null allele onto BXSB-Yaa mice. (C) Kaplan–Meir survival analysis of *Il21r*^{+/−} vs. *Il21r*^{−/−} mice after 11 generations of backcross, $P < 0.0001$.

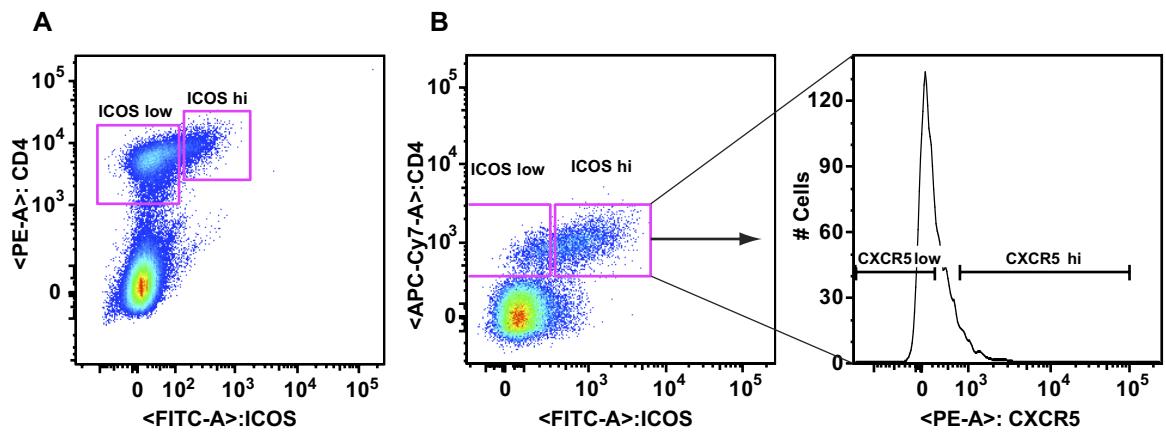


Fig. S4. Flow cytometric gates for isolation BXSB-Yaa CD4 T cells for gene expression analysis. (A) Isolation of ICOS^{hi} and ICOS^{lo} CD4⁺ T cells for Fig. 5D *Upper*. (B) Isolation of ICOS^{hi} CXCR5^{hi} and ICOS^{hi} CXCR5^{lo} CD4⁺ T cells for Fig. 5D *Lower*.

Table S1. Activation markers on BXSB-Yaa lymphocyte populations

Mice	Fluorescence intensity			
	B cells		T cells	
	Ly6a/e	CD69	Ly6a/e	CD69
<i>Il21r</i> ^{+/-}	484.2 ± 6.5	243.0 ± 7.9	563.8 ± 11.0	279.0 ± 11.3
<i>Il21r</i> ^{-/-}	377.3 ± 6.7	181.5 ± 2.7	371 ± 6.3	182.3 ± 2.5
P value	<0.0001	<0.0001	<0.0001	<0.0001

Values are mean ± SEM of 10 16-week-old mice.

Table S2. Genes and oligonucleotide primers used for qPCR in Figs. 5 and S2

Gene	Forward	Reverse
<i>Cd83</i>	CTGATGTGCCCTGGCTTG	CAAACATTGGGGCCAGTGA
<i>Cxcr4</i>	ATCAGCTGGACCGGTACCT	GCAGTTCTTGGCCTTGAC
<i>Cxcr5</i>	CGGAGCTCAACCGAGACCTT	CCACTGAAAAGGCAGGATG
<i>IgG1</i>	CACCTCCAAGGAGCAGATG	CCCAGTTGCTCTTCGACAT
<i>IgG2b</i>	CATCACCATCGAGAGAACCA	ACACTGATGTCTCAGGGTTGA
<i>Il10</i>	CAGAGAAGCATGGCCAGAA	GTCAAATTCTTATGGCCTTGT
<i>Il17a</i>	GAAGATGCTGGGGTGTGG	AGCCCGGGTCTGTTAG
<i>Il17b</i>	TGGAAGAGTATGAGCGGAAC	ATTCAACGCAACCCAAACATA
<i>Il17c</i>	TCTCCTGCTTCTAGGCTGGT	GCACCTCGAGTTAGCAGGTG
<i>Il17d</i>	ATCACACACATCCCGTTTC	GAGACCTTAGGTGCCAGA
<i>Il17f</i>	CTCCCCTGGAGGATAACACT	GACCAGGATTCTTGCTGAA
<i>IL-21</i>	CCTGGAGTGGTATCATCGCTT	TGATTGTGACACTTCTGGGAAT
<i>Il21r</i>	GTGACCCCGTCATTTCAGA	CCGCTGTGCTCCCTGTACA
<i>Psgl1</i>	CCACCATTTCTCGTGTGC	GGGGAGTAGTTCCGCACTGG